

REMARKS

In this response to the Final Office Action dated December 9, 2009, Claim 1 has been amended to incorporate the features of Claim 29. Claim 10 has been amended to correct a spelling error and further support for the amendment can be found, for example, from page 3, lines 12-14 of the originally filed specification. Claim 12 has been amended to be dependent from Claim 1. Claim 13 has been amended to remove "intraperitoneal injection". Claim 28 has been amended to recite "neurons" and support for the amendment can be found, for example, from page 4, lines 6-7 of the specification. Claims 4 and 29 have been canceled without prejudice. New Claims 30-34 have been added. Support for the new Claim 30 can be found, for example, from page 3, lines 6, 8-9, 12-14, and 17-18, page 7, line 14, page 8, line 12 as well as Examples 1-4 and Figures 1-17 of the specification. Support for the new Claims 31-34 can be found, for example, from Claims 14-17 as originally filed. No new matter is added in these amendments.

Claims 1, 3, 5-17, 28, and 30-34 are currently pending in the application. In view of the amendments and remarks as set forth herein, Applicants respectively request withdrawal of the claim rejections and reconsideration of the pending claims.

Allowable subject matter

Applicants thank the Examiner for notifying that Claim 29 is patentable over the prior art and thus would be in condition of allowance if written in independent form.

Claim rejections by under 35 U.S.C 102(b)

Claims 1, 4, and 13 were rejected under 35 U.S.C. 102(b) as anticipated by Penkowa *et al.* (2002, Journal of Comparative Neurology 444(2): 174-189). In order to expedite the prosecution, Applicants have amended Claim 1 to incorporate the features of Claim 29. As correctly acknowledged by the Examiner, Claim 29 is patentable over the prior art including Penkowa and thus Claim 1, which now recites the features of Claim 29, should also be patentable. Reconsideration of Claim 1 in light of the amendment is respectfully requested.

As Claim 4 has been canceled, the rejection to the claim is now moot.

Claim 13 has been amended to remove "intraperitoneal injection" and is dependent from Claim 1. Through its dependency, Claim 13 incorporates all the features of Claim 1. As such, Claim 13 should also be patentable for at least the same reasons that Claim 1 is patentable. Reconsideration of Claim 13 is respectfully requested.

Claims 1, 4, 13, and 17 were rejected under 35 U.S.C. 102(b) as anticipated by Giralt *et al.* (2002, *Experimental Neurology* 173: 114-128). In order to expedite the prosecution, Applicants have amended Claim 1 as set forth above. In light of the amendment, Claims 1 is in condition of immediate allowance. Reconsideration of Claim 1 is respectfully requested.

As Claim 4 has been canceled, the rejection to the claim is now moot. As to Claims 13 and 17, they incorporate all the features of Claim 1, through their ultimate dependencies from Claim 1. As such, Applicants respectfully subject that Claims 13 and 17 are also allowable and thus reconsideration of the claims is requested.

Claim rejections under 35 U.S.C. 103(a)

In the section of rejection under 35 U.S.C. 103(a), the Examiner rejected Claims 1, 4, and 6-13 as being unpatentable over Penkowa; rejected Claims 1 and 3-13 as being unpatentable over Penkowa in view of FR 2813529; rejected Claims 1, 4, 6-13, and 28 as being unpatentable over Penkowa in view of Deguchi (2000, *Pharmaceutical Research* 17(1): 63-69) and Yoshimura (2001, *Proc Natl Acad Sci USA* 98(10): 5874-5879); rejected Claims 1, 4, 6-13, 15, and 28 as being unpatentable over Penkowa in view of Deguchi and Yoshimura, and further in view of Asanuma (2002, *Neurosciences Letters* 327:61-65); and rejected Claims 1, 4, 6-14, 16, and 28 as being unpatentable over Penkowa in view of Deguchi, Yoshimura, and in further view of Walsh (US Patent Application publication 2002/0155170). Applicants respectfully traverse the foregoing rejections.

As noted above, Applicants have amended Claim 1 to incorporate the features of Claim 29, which is patentable over the prior art. In light of this amendment, Claim 1 is now in condition of immediate allowance over the prior art. For at least the same reasons that Claim 1 is patentable, its dependent claims, namely Claims 3, 6-16, and 28, which incorporate all the features of Claim 1, are also in condition of allowance. Accordingly, withdrawal of the rejection to Claims 1, 3, 6-16, and 28 is respectfully requested. As Claim 4 has been canceled, the rejection to the claim is now moot.

Patentability of new Claims 30-34

New Claim 30 recites, among others, a method of stimulating outgrowth of neurites comprising directly contacting a target living neuron or live neuronal area with a solution of a metallothionein isoform selected from the group consisting of MT-I, MT-II and synthetic

forms thereof so as to deliver a sufficient amount of said metallothionein to stimulate said outgrowth of neurites. Applicants respectfully submit that new Claim 30 is patentable over the prior art.

Claim 30 is patentable over Penkowa

In the previous responses, Applicants described the deficiencies of Penkowa in detail. For example, Penkowa may teach the neuroprotective capacity of metallothionein but clearly fails to teach regenerative growth of neurons. Further, Penkowa method would be unable to provide sufficient metallothionein to stimulate outgrowth of neurites. As presented in the Declaration filed on August 20, 2009, Applicants administered metallothionein in a 50 fold higher dose than as originally stated in Penkowa to mice under the conditions as described by the reference. When monitored in mice brain after 40 minutes of administration, the actually delivered amount of metallothionein by Penkowa method would be much less than 0.016 $\mu\text{g/g}$ tissue wet weight, which is a clearly negative result.

However, in the outstanding office action, the Examiner asserted that the declaration is insufficient to overcome the rejection based upon anticipation by Penkowa. The Examiner further stated that if Applicants would provide evidence that four days after MT-2 peripheral administration this protein is not found in the brain, such evidence may be sufficient to overcome the rejection over Penkowa for anticipation. In reply, Applicants further provide the experimental data showing that even after 4 days, metallothionein administered according to Penkowa method was not found in the tested mice brain tissue. See the accompanying Declaration under 37 CFR 1.132.

In the accompanying Declaration, the experimental protocols used to produce these data are similar to those described in the previously submitted Declaration. Applicants have administered rabbit Zn-MT2A by intraperitoneal (i.p.) injection immediately (within three minutes) following cryolesion. Transgenic mice (seven adult male mice in total) lacking MT-1 and MT-2 genes were employed. The mice received daily doses of 175 μg MT-2 over four days. The dose equates to more than 10X the dose administered by Penkowa. Mice were harvested 4 days (three mice) post lesion. One mouse was left unwounded and uninjected as an experimental control. Tissue containing the lesion was removed from each injured mouse, and protein samples were prepared and analyzed by Western blotting using antibody to detect MT-2. This method has proven to be routinely quantitative, highly sensitive and unambiguous. Applicants did not observe any protein bands (white lines) in the 7-12 kDa

size range (the size of MT-2) in the brains of any of the mice that received MT-2 injections. See Figure 1 (Exhibits A).

A calibration Western Blot was performed under the same conditions using known amounts of MT-2 to determine the sensitivity of the assay. As shown in Figure 2 (Exhibit B), the method allows for detection of down to 8ng MT-2. As 3.3ug total protein was loaded onto each lane of the western blot gel, this indicates that there was less than 3µg MT-2 per mg of protein in the tissue samples. The fact that Applicants have observed no MT-2 in the brain of MT-knockout mice that have received a cortical cryolesion and been subsequently injected i.p. with MT-2 at 10X the amount disclosed by Penkowa, proves that MT-2 is not reaching the injured brain at levels required for promoting nerve regeneration.

In light of the foregoing evidence, Applicants believe Penkowa cannot and does not anticipate the subject matter of Claim 30.

In addition, Applicants respectfully submit that Claim 30 would be non-obvious over Penkowa reference. As mentioned, Penkowa is silent in teaching of regenerative growth of neurons and thus it fails to teach the claimed method, which is related to stimulating outgrowth of neurites. Further, as experimentally shown in the Inventor's accompanying Declaration, Penkowa method is unable to deliver a sufficient amount of a metallothionein (or its isoform selected from the group consisting of MT-I, MT-II and synthetic forms thereof) to stimulate said outgrowth of neurites. In addition, Penkowa method is intraperitoneal injection, in other words, a peripheral administration. In contrast, the method according to Claim 30 is configured to directly contact a target living neuron or live neuronal area with a solution of a metallothionein (or its isoform).

As such, Penkowa does not and cannot teach or suggest the limitations of Claim 30. In addition, none of the cited references, namely FR 2813529, Deguchi, Yoshimura, Asanuma, and Walsh, does remedy the foregoing deficiencies of Penkowa. As such, Claim 30 would be non-obvious and patentable over all of the foregoing references. For at least the same reasons that Claim 30 is patentable over the prior art, its dependent Claims 31-34 would be also patentable. Favorable consideration of Claims 31-34 is respectfully requested.

Claim 30 is patentable over Giralt

Applicants respectfully submit that Claim 30 would not be anticipated or obvious over Giralt reference alone or any combinations of Giralt and other references. Similar to Penkowa, Giralt appears to teach administration of MT-2 to mice by intraperitoneal injection.

In the Giralt method, the administration of MT-2 may be done for three or seven days (See page 115, first column, last paragraph of Giralt). In the last Declaration filed August 20, 2009, Applicants also provided experimental evidence showing that Giralt method is unable to stimulate neurite outgrowth as recited in Claim 30. As done to Penkowa method, a 50-fold higher dose than as originally stated in Giralt was administered to mice as described in Giralt, however no noticeable level of metallothionein was detected in the neuronal tissue (i.e. Brain, 40 minutes after administration).

To further support the foregoing deficiency of Giralt method, Applicants provide additional experimental data in the accompanying Declaration. Applicants tested the dose more than 5X the dose administered by Giralt to six mice and tested the delivery of MT-2 in the brain 7 days after i.p. injection of MT-2. As clearly shown in Figure1 (Exhibit A), the Giralt method was unable to deliver MT-2 in the injured brain at levels required for promoting nerve regeneration. As such, Giralt also does not and cannot teach or suggest the method of Claim 30. Accordingly, Applicants respectfully submit that new Claim 30 is patentable over Giralt.

In light of the foregoing remarks and the data presented in the accompanying Declaration, Applicants respectfully submit that Claim 30 and its dependent Claims 31-34 would be patentable over the prior art and thus request to allow the claim.

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, Applicants are not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. Applicants reserve the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that Applicants have made any disclaimers or disavowals of any subject matter supported by the present application.

CONCLUSION

Applicants have endeavored to address all of the Examiner's concerns as expressed in the outstanding Office Action. Accordingly, arguments in support of the patentability of the pending claim set are presented above.

In light of the above remarks, reconsideration and withdrawal of the outstanding rejections is respectfully requested. If the Examiner has any questions which may be answered by telephone, he is invited to call the undersigned directly.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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